Amendments to the Specification:

Please insert the following new paragraph on page 1 of the specification following the title:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of Application No. 09/409,926, filed September 30, 1999, now U.S. Patent No. 6,617,442.

Please insert the Sequence Listing being filed concurrently herewith into the specification.

Please replace the paragraph on page 9, lines 10 through 19, with the following rewritten paragraph:

Thus, in accordance with one aspect of the present invention, there are provided isolated polynucleotides which encode human Type 2 RNase H polypeptides having the deduced amino acid sequence of ~~Figure 1~~ Figure 6. By "polynucleotides" it is meant to include any form of RNA or DNA such as mRNA or cDNA or genomic DNA, respectively, obtained by cloning or produced synthetically by well known chemical techniques. DNA may be double- or single-stranded. Single-stranded DNA may comprise the coding or sense strand or the non-coding or antisense strand.

Please replace the paragraph on page 9, lines 20 through 35, and continuing on page 10, lines 1 through 3, with the following rewritten paragraph:

Methods of isolating a polynucleotide of the present invention via cloning techniques are well known. For example, to obtain the cDNA contained in ATCC Deposit No. 98536, primers based on a search of the XREF database were used. An approximately 1 Kb cDNA corresponding to the carboxy terminal portion of the protein was cloned by 3' RACE. Seven positive clones were isolated by screening a liver cDNA library with this 1 Kb cDNA. The two longest clones were 1698 and 1168 base pairs. They share the same 5' untranslated region and protein coding sequence but differ in the length of the 3' UTR. A single reading

frame encoding a 286 amino acid protein (calculated mass: 32029.04 Da) was identified ~~(Figure 1)~~ (Figure 6). The proposed initiation codon is in agreement with the mammalian translation initiation consensus sequence described by Kozak, M., J. Cell Biol., 1989, 108, 229-241, and is preceded by an in-frame stop codon. Efforts to clone cDNA's with longer 5' UTR's from both human liver and lymphocyte cDNA's by 5' RACE failed, indicating that the 1698-base-pair clone was full length.

Please replace the paragraph on page 10, lines 4 through 27, with the following rewritten paragraph:

In a preferred embodiment, the polynucleotide of the present invention comprises the nucleic acid sequence of the cDNA contained within ATCC Deposit No. 98536. The deposit of E. coli DH5α containing a BLUESCRIPT® plasmid containing a human Type 2 RNase H cDNA was made with the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, USA, on September 4, 1997 and assigned ATCC Deposit No. 98536. The deposited material is a culture of E. coli DH5α containing a BLUESCRIPT® plasmid (Stratagene, La Jolla CA) that contains the full-length human Type 2 RNase H cDNA. The deposit has been made under the terms of the Budapest Treaty on the international recognition of the deposit of micro-organisms for the purposes of patent procedure. The culture will be released to the public, irrevocably and without restriction to the public upon issuance of this patent. The sequence of the polynucleotide contained in the deposited material and the amino acid sequence of the polypeptide encoded thereby are controlling in the event of any conflict with the sequences provided herein. However, as will be obvious to those of skill in the art upon this disclosure, due to the degeneracy of the genetic code, polynucleotides of the present invention may comprise other nucleic acid sequences encoding the polypeptide of ~~Figure 1~~ Figure 6 and derivatives, variants or active fragments thereof.

Please replace the paragraph on page 10, lines 28 through 35, and continuing on page 11, lines 1 through 10, with the following rewritten paragraph:

Another aspect of the present invention relates to the polypeptides encoded by the polynucleotides of the present invention. In a preferred embodiment, a polypeptide of the present invention comprises the deduced amino acid sequence of human Type 2 RNase H provided in ~~Figure 1~~ Figure 6 as SEQ ID NO: 1. However, by "polypeptide" it is also meant to include fragments, derivatives and analogs of SEQ ID NO: 1 which retain essentially the same biological activity and/or function as human Type 2 RNase H. Alternatively, polypeptides of the present invention may retain their ability to bind to an antisense-RNA duplex even though they do not function as active RNase H enzymes in other capacities. In another embodiment, polypeptides of the present invention may retain nuclease activity but without specificity for the RNA portion of an RNA/DNA duplex. Polypeptides of the present invention include recombinant polypeptides, isolated natural polypeptides and synthetic polypeptides, and fragments thereof which retain one or more of the activities described above.

Please replace the paragraph on page 11, lines 11 through 34, with the following rewritten paragraph:

In a preferred embodiment, the polypeptide is prepared recombinantly, most preferably from the culture of E. coli of ATCC Deposit No. 98536. Recombinant human RNase H fused to histidine codons (his-tag; in the present embodiment six histidine codons were used) expressed in E.coli can be conveniently purified to electrophoretic homogeneity by chromatography with Ni-NTA followed by C4 reverse phase HPLC. The purified recombinant polypeptide of SEQ ID NO: 1 is highly homologous to E.coli RNase H, displaying nearly 34% amino acid identity with E.coli RNase H1. ~~Figure 1~~ Figure 6 compares the protein sequences deduced from human RNase H cDNA (SEQ ID NO: 1) with those of chicken (SEQ ID NO: 2), yeast (SEQ ID NO: 3) and E.coli RNase HI (Gene Bank accession no. 1786408; SEQ ID NO: 4), as well as an EST deduced mouse RNase H homolog (Gene Bank accession no. AA389926 and AA518920; SEQ ID NO: 5). The deduced amino acid sequence of human RNase H (SEQ ID NO: 1) displays strong homology with yeast (21.8% amino acid identity), chicken (59%), E.coli RNase HI (33.6%) and the mouse EST homolog (84.3%). They are all small proteins (<40 KDa) and their estimated pIs are all 8.7

and greater. Further, the amino acid residues in E.coli RNase HI thought to be involved in the Mg2+ binding site, catalytic center and substrate binding region are completely conserved in the cloned human RNase H sequence ~~(Figure 1)~~ Figure 6.

Please replace the paragraph on page 27, lines 10 through 17, with the following rewritten paragraph:

2'-*O*-MOE r(CGCGAAUUCGCG) ~~SEQ ID NO: 1~~ SEQ ID NO.: 72 was synthesized, crystallized and its structure at a resolution of 1.7 Ångstrom was determined. The crystallization conditions used were 2 mM oligonucleotide, 50 mM Na Hepes pH 6.2-7.5, 10.50 mM $MgCl_2$, 15% PEG 400. The crystal data showed: space group *C*2, cell constants *a*=41.2 Å, *b*=34.4 Å, *c*=46.6 Å, β=92.4°. The resolution was 1.7 Å at -170°C. The current *R*=factor was 20% (R_{free} 26%).

Please replace the paragraph on page 29, lines 13 through 24, with the following rewritten paragraph:

In extending the crystallographic structure studies, molecular modeling experiments were performed to study further enhanced binding affinity of oligonucleotides having 2'-O-modifications of the invention. The computer simulations were conducted on compounds of ~~SEQ ID NO: 1~~ SEQ ID NO: 72, above, having 2'-O-modifications of the invention located at each of the nucleoside of the oligonucleotide. The simulations were performed with the oligonucleotide in aqueous solution using the AMBER force field method (Cornell et al., J. Am. Chem. Soc., **1995**, 117, 5179-5197) (modeling software package from UCSF, San Francisco, CA). The calculations were performed on an Indigo2 SGI machine (Silicon Graphics, Mountain View, CA).

Please replace the paragraph on page 82, Table 1, lines 5-20, with the following rewritten paragraph:

Table I

3'-O-(2-methoxyethyl) containing 2'-5' linked oligonucleotides.

ISIS #	Sequence (5'-3')1	Backbone	Chemistry
17176	ATG-CAT-TCT-GCC-CCC-AAG-GA* (SEQ ID NO.: 13)	P=S	3'-O-MOE
17177	ATG-CAT-TCT-GCC-CCC-AAG-G*A* (SEQ ID NO.: 14)	P=S	3'-O-MOE
17178	ATG-CAT-TCT-GCC-CCC-AAG$_O$-G*$_O$A* (SEQ ID NO.: 15)	P=S/P=O	3'-O-MOE
17179	A*TG-CAT-TCT-GCC-CCC-AAG-GA* (SEQ ID NO.: 16)	P=S	3'-O-MOE
17180	A*TG-CAT-TCT-GCC-CCC-AAG-G*A* (SEQ ID NO.: 17)	P=S	3'-O-MOE
17181	A*$_O$TG-CAT-TCT-GCC-AAA-AAG$_O$-G*$_O$A* (SEQ ID NO.: 18)	P=S/P=O	3'-O-MOE
21415	A*T*G-CAT-TCT-GCC-AAA-AAG-G*A* (SEQ ID NO.: 19)	P=S	3'-O-MOE
21416	A*$_O$T*$_O$G-CAT-TCT-GCC-AAA-AAG$_O$-G*$_O$A* (SEQ ID NO.: 20)	P=S/P=O	3'-O-MOE
21945	A*A*A*	P=O	3'-O-MOE
21663	A*A*A*A*	P=O	3'-O-MOE
20389	A*U*C*G*	P=O	3'-O-MOE
20390	C*G*C*-G*A*A*-T*T*C*-G*C*G* (SEQ ID NO.: 21)	P=O	3'-O-MOE

Please replace the paragraph on page 85, Table III, lines 19-32, with the following rewritten paragraph:

Table III
Tm Analysis of Oligonucleotides

ISIS #	Sequence (5'-3')	Backbone	T_m	# Mods	#2'-5' Linkages
11061	ATG-CAT-TCT-GCC-CCC-AAG-GA (SEQ ID NO.: 22)	P=S	61.4	0	0
17176	ATG-CAT-TCT-GCC-CCC-AAG-GA* (SEQ ID NO.: 13)	P=S	61.4	1	0
17177	ATG-CAT-TCT-GCC-CCC-AAG-G*A* (SEQ ID NO.: 14)	P=S	61.3	2	1
17178	ATG-CAT-TCT-GCC-CCC-AAG$_O$-G*$_O$A* (SEQ ID NO.: 15)	P=S/P=O	61.8	2	1
17179	A*TG-CAT-TCT-GCC-CCC-AAG-GA* (SEQ ID NO.: 16)	P=S	61.1	2	1
17180	A*TG-CAT-TCT-GCC-CCC-AAG-G*A* (SEQ ID NO.: 17)	P=S	61.0	3	2
17181	A*$_O$TG-CAT-TCT-GCC-AAA-AAG$_O$-G*$_O$A* (SEQ ID NO.: 18)	P=S/P=O	61.8	3	2
21415	A*T*G-CAT-TCT-GCC-AAA-AAG-G*A* (SEQ ID NO.: 19)	P=S	61.4	4	3
21416	A*$_O$T*$_O$G-CAT-TCT-GCC-AAA-AAG$_O$-G*$_O$A* (SEQ ID NO.: 20)	P=S/P=O	61.7	4	3

Please replace the paragraph beginning on page 86, lines 29-33, and continuing on page 87, lines 1-11, with the following rewritten paragraph:

Thermal melts were done as per standarized literature procedures. Oligonucleotide identity is as follows: Oligonucleotide A is a normal 3'-5' linked phosphodiester oligodeoxyribonucleotide of the sequence d(GGC TGU* CTG CG) (SEQ ID NO.: 23) where the * indicates the attachment site of a 2'-aminolinker. Oligonucleotide B is a normal 3'-5' linked phosphodiester oligoribonucleotide of the sequence d(GGC TGU* CTG CG) (SEQ ID NO.: 23) where the * indicates the attachment site of a 2'-aminolinker. Each of the ribonucleotides of the oligonucleotide, except the one bearing the * substituent, are 2'-O-methyl ribonucleotides. Oligonucleotide C has 2'-5' linkage at the * position in addition to a 3'-aminolinker at this site. The remainder of the oligonucleotide is a phosphodiester oligodeoxyribonucleotide of the sequence d(GGC TGU* CTG CG) (SEQ ID NO.: 23). The base oligonucleotide (no 2'-aminolinker) was not included in the study.

Please replace the paragraph on page 88, Table IV, lines 1-10, with the following rewritten paragraph:

Table IV

Modified Oligonucleotides

synthesized to evaluate stability

ISIS #	Sequence (5'-3')	Backbone	Chemistry
22110	TTT-TTT-TTT-TTT-TTT-**T*T*T*-T*** (SEQ ID NO.: 24)	P=O	3'-*O*-MOE
22111	TTT-TTT-TTT-TTT-TTT-**T#T#T#-U#** (SEQ ID NO.: 25)	P=O	3'-*O*-MOE
22112	TTT-TTT-TTT-TTT-TTT-**T*T*T*-T*** (SEQ ID NO.: 26)	P=S	3'-*O*-MOE
22113	TTT-TTT-TTT-TTT-TTT-**T#T#T#-U#** (SEQ ID NO.: 27)	P=S	3'-*O*-MOE
22114	TTT-TTT-TTT-TTT-TTT_O-**T*$_O$T*$_O$T*$_O$T*** (SEQ ID NO.: 28)	P=S/P=O	3'-*O*-MOE
22115	TTT-TTT-TTT-TTT-TTT_O-**T#$_O$T#$_O$T#$_O$-U#** (SEQ ID NO.: 29)	P=S/P=O	3'-*O*-MOE

Please replace the paragraph on page 88, Table V, lines 16-26, with the following rewritten paragraph:

Table V

Properties of Modified Oligonucleotides

ISIS	#Sequence (5'-3')[1]	Expected	Observed	HPLC[2]	Ods(260nm)
Mass	Mass (min.)	T_R Purified			
22110	TTT-TTT-TTT-TTT-TTT-**T*T*T*T***-T* (SEQ ID NO: 24)	6314.189	6315.880	20.39	174
22111	TTT-TTT-TTT-TTT-TTT-**T#T#T#T#**-U# (SEQ ID NO: 25)	6004.777	5997.490	20.89	147
22112	TTT-TTT-TTT-TTT-TTT-**T*T*T*T***-T* (SEQ ID NO: 26)	6298.799	6301.730	25.92	224
22113	TTT-TTT-TTT-TTT-TTT-**T#T#T#T#**-U# (SEQ ID NO: 27)	6288.745	6286.940	24.77	209
22114	TTT-TTT-TTT-TTT-TTT$_O$-**T*$_O$T*$_O$T*$_O$T***-T* (SEQ ID NO: 28)	6234.799	6237.150	24.84	196
22115	TTT-TTT-TTT-TTT-TTT$_O$-**T#$_O$T#$_O$T#$_O$T#**-U# (SEQ ID NO: 29)	6224.745	6223.780	23.30	340

Please replace the paragraph on page 90, Table VI, lines 10-19, with the following rewritten paragraph:

Table VI

Oligonucleotides bearing Aminopropyl Substituents

ISIS #	Sequence (5'-3')[1]	Backbone
23185-1	A*TG-CAT-TCT-GCC-CCC-AAG-GA* (SEQ ID NO.: 30)	P=S
23186-1	A*TG-CAT-TCT-GCC-CCC-AAG-GA* (SEQ ID NO.: 31)	P=S
23187-1	A*$_o$T$_o$G$_o$-C$_o$A$_s$T$_s$-T$_s$C$_s$T$_s$-G$_s$C$_s$C$_s$-C$_s$C$_s$C$_s$-A$_o$A$_o$G$_o$-G$_o$A* (SEQ ID NO.: 32)	P=S/P=O
23980-1	*A**$_o$T$_o$G$_o$-C$_o$A$_s$T$_s$-T$_s$C$_s$T$_s$-G$_s$C$_s$C$_s$-C$_s$C$_s$C$_s$-A$_o$A$_o$G$_o$-G$_o$*A** (SEQ ID NO.: 33)	P=S/P=O
23981-1	*A**TG-CAT-TCT-GCC-CCC-AAG-G*A** (SEQ ID NO.: 34)	P=S
23982-1	A*TG-CAT-TCT-GCC-CCC-AAG-GA* (SEQ ID NO.: 35)	P=S

Please replace the paragraph on page 97, Table VIII, lines 1-11, with the following rewritten paragraph:

Table VIII

ICAM-1 Oligonucleotides Containing MMI Dimers Synthesized for in Vivo Nuclease and Pharmacology Studies.

ISIS #	Sequence (5'-3')	Backbone	2'-Chemistry
16314	TGC ATC CCC CAG GCC ACC A*T (SEQ ID NO.: 36)	P=S, MMI	Bis-2'-OMe-MMI, 2'-H
16315	T*GC ATC CCC CAG GCC ACC A*T (SEQ ID NO.: 37)	P=S, MMI	Bis-2'-OMe-MMI, 2'-H
3082 mismatch	TGC ATC CCC CAG GCG ACC AT (SEQ ID NO.: 38)	P=S	2'-H, single
13001	TGC ATC CCC CAG GCC ACC AT (SEQ ID NO.: 39)	P=S	2'-H

Please replace the paragraph on page 97, Table IX, lines 14-23, with the following rewritten paragraph:

Table IX

Physical Characteristics of MMI Oligomers Synthesized for Pharmacology, and In Vivo Nuclease Studies

ISIS # Retn. (min)	Sequence (5'-3')	Expected Mass (g)	Observed Mass (g)	HPLC Time
16314	TGC ATC CCC CAG GCC ACC A*T (SEQ ID NO.: 36)	6295	6297	23.9
16315	T*G C ATC CCC CAG GCC ACC A*T (SEQ ID NO.: 37)	6302	6303	24.75

Please replace the paragraph on page 129, lines 4-17, with the following rewritten paragraph:

Substrate duplexes were hybridized and initial rates were determined as shown in Table 4 and described in Material and Methods. The 17mer RNA is the same used in Table 4, and the 20mer RNA (UGGUGGGCAAUGGGCGUGUU, RNA no.4) (SEQ ID NO.: 40) is derived from the protein kinase C-zeta (53) sequence. The 17mer and 20mer P=S oligonucleotides were full deoxyphosphorothioate containing no 2'-modifications. The 9, 7, 5, 4 and 3 deoxy gap oligonucleotides were 17mer oligonucleotide with a central portion consisting of nine, seven, five and four deoxynucleotides flanked on both sides by 2'-~~methoexynucleotides~~ methoxynucleotides (also see Figure 2). Boxed sequences indicate the position of the 2'-methoxy-modified residues. Dash-boxed sequence indicates the position of the 2'-propoxy-modified residues.

Please replace Table 4, on page 131, with the following rewritten Table 4:

Table 4

A

	RNA	*Antisense*	*Initial Rate*
1	GGGCGCCGUCGGUGU GG (SEQ ID NO.: 41)	17mer P=O	1050±203
1	GGGCGCCGUCGGUGU GG (SEQ ID NO.: 42)	17mer P=S	4034±266

B

RNA No.	*RNA*	*Antisense DNA*	*Initial Rate* (pmol $L^{-1}min^{-1}$)
1	GGGCGCCGUCGGUGUGG (SEQ ID NO.: 41)	17mer P=O	1050±203
2	ACUCCACCAUAGUACACUCC (SEQ ID NO.: 59)	20mer P=O	1015±264
3	UGGUGGGCGCCGUCGGUGUGGGCAA (SEQ ID NO.: 60)	25mer P=O	1502±182

Please replace Table 5, on page 132, with the following rewritten Table 5:

Table 5

RNA No.	*RNA*	*Antisense DNA*	*Initial Rate (pmol $L^{-1}min^{-1}$)*
1	17mer	CCACACCGACGGCGCCC (SEQ ID NO.: 61)	4034±266
	17mer	CCACACCGACGGCGCCC (SEQ ID NO.: 61)	1081±168
	17mer	CCACACCGACGGCGCCC (SEQ ID NO.: 61)	605±81
	17mer	CCACACCGACGGCGCCC (SEQ ID NO.: 61)	330±56
	17mer	CCACACCGACGGCGCCC (SEQ ID NO.: 61)	0
	17mer	CCACACCGACGGCGCCC (SEQ ID NO.: 61)	0
	17mer	CCACACCGACGGCGCCC (SEQ ID NO.: 61)	0
4*	20mer	AACACGCCCATTGCCCACCA (SEQ ID NO.: 62)	3400±384
	20mer	ACAACGCCCATTGCCCACCA (SEQ ID NO.: 62)	0

*Table legend for sequence